The potential use of the bivalve *Donax trunculus* as Bio-indicator for heavy metal pollution of Port Said western coast on the Mediterranean Sea

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Abstract: Heavy metals were determined in the most abundant species of bivalve mollusks (*Donax trunculus*) along Port Said coasts on southeastern Mediterranean Sea and in the sediments where they live. The mean concentrations ($\mu g g^{-1}$) of heavy metals in bottom sediment were as follows: Fe: 1748.2-1918.7, Mn: 191.4-217.8, Zn: 28-36.6, Cu: 5.7-9.4, Pb: 18.8-24.4, Cd: 1.4-2.0, and in surface water: Fe: 822.6-896, Mn: 169.3-198, Zn: 271.3.6-300, Cu: 12.6-19.3, Pb: 40.0-56.0, Cd: 0.8-2.7, while in soft tissues of the bivalve *D. trunculus*, the mean concentrations ($\mu g g^{-1}$ dry weight) were as follows: Fe: 57.2-66.4, Mn: 6.0-7.6, Zn: 32.8-36.4, Cu: 4.0-4.4, Pb: 8.8-9.2, Cd: 2.1-2.4. The present study has revealed that the concentrations for human consumption proposed by FAO/WHO, EU. Moreover, estimation of concentration factor (C_f) for the studied metals in the soft tissues of the edible bivalve *D. trunculus* recorded high accumulation rates of Cd and Cu. The present study confirmed that, the examined bivalve species was associated with enhanced metal content in its soft tissues and was not safe within the limits for human consumption. The potential use of this bivalve species as a suitable bio-indicator was evaluated from correlation tests based on the concentrations of heavy elements in the sediment-metals system and in the water-metals system to those in the bivalve.

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1. Introduction

Heavy metals from seawater and marine sediments are known to be accumulated by many species of marine invertebrates such as oysters, mussels, clams and shells. Their usefulness as bioindicator organisms provides, ideally, an estimate of trace elements availabilities to the biomass of different areas and localities (Yusof et al., 2004). Bivalve mollusks have been used to assess the levels of contamination in marine ecosystems, and certain genera and species, notably mussels and ovsters, have been extensively studied in temperate waters (Paez-Osuna et al., 1993). According to Saiz-Salinas et al. (1996) the contamination of these organisms provides a time integrated measure of metal bioavailability, responding essentially to that fraction of the total environmental load which is of direct ecotoxicological relevance (Phillips and Seger, 1986; Rainbow and Phillips, 1993).

Heavy metals are considered among most

serious contaminants of aquatic ecosystems, due to their high potential to enter and accumulate in food chain (Tam and Wong, 2000; Erdoğrul and Erbilir, 2007). Under certain environmental conditions in aquatic systems, heavy metals may accumulate to reach a toxic concentration and cause ecological damage (Jefferies and Firestone, 1984; Freedman, 1989). The main sources of heavy metal pollution are (1) the run-off from agricultural and urban areas, (2) discharges from mining, factories and municipal sewer systems, (3) leaching from dumps and former industrial sites, and (4) atmospheric deposition (Singh and Steinnes, 1994; Kumar Singh et al., 2007).

Coastal areas are characterized by high organic matter and nutrients from the continent, having fragile coastal ecosystem dependent on terrestrial conditions (Yáñez-Aracibia and Sánchez-Gil, 1988). Industrialization of coastal areas is very common in countries characterized by exploitation and importation economics, causing serious damage to coastal ecosystems, e.g. contamination of metals (Cardoso et al., 2001). Moreover, anthropogenic activities are known to have a wide range of potential effects of these coastal ecosystems, particularly from point and non-point sources of pollution. The release of pollutants into coastal environment is a major human concern worldwide. These contaminants are known to readily accumulate in bottom sediments which serve as a repository of pollutants. Sediment contaminants could be released to the overlying water, resulting in potential adverse health effects to aquatic organisms (Daskalakis and O'Connor, 1995; Long et al., 1995; Argese et al., 1997; Ross and Delorenzo, 1997; Freret-Meurer et al., 2010). Among the adverse health effects associated with these contaminants are toxicity to the kidney, nervous and reproductive systems, as well as endocrine disruption and mutations (Collier et al., 1998; Nirmala et al., 1999; Ketata et al., 2007; Liu et al., 2008; Brar et al., 2009). In addition, trace metals are known to bioaccumulate in edible aquatic organisms (e.g., mollusks), thus, representing a health risk to top predators, including humans (Fox et al., 1991; Renzoni et al., 1998; Huang et al., 2006; Díez et al., 2009).

The Egyptian coastline extends 3000 km along the Mediterranean Sea and Red Sea beaches in addition to the Gulf of Suez and Gulf of Aqaba. Unfortunately, most of the Egyptian coastal zones along the Mediterranean Sea are subjected to intense discharges of pollutants from numerous anthropogenic activities (Dowidar, 1988). A high profile example is the region of El-Gamil coast at the northwestern part of Port Said City (Kaiser et al., 2010). Contamination by trace metals has not been extensively studied in the Egyptian coastal zones along the Mediterranean Sea which are subjected to intense discharges of pollutants (Hamed, 1996; El-Sikaily, et al., 2004; El-Moselhy, et al., 2005). Moreover, some alarm has been expressed problems concerning possible health related associated with seafood consumption due to the presence of certain heavy metals in quantities exceeding those of the maximum permeable limit (MPL) allowed under the standards.

This study attempts to look at the distribution and dispersion of some heavy metals namely Fe, Mn, Zn, Cu, Pb and Cd using the bivalve (*Donax trunculus*) as the bio-indicator since individuals of this species accumulate heavy metals from water, sediments and/or from food. So, this study aims to provide basic information for detecting the current status of heavy metal pollution in Port Said zone of the Egyptian coasts on southeastern Mediterranean Sea, and to determine if humans that consume the bivalve *Donax trunculus* from this region might be at risk for heavy metal-related health issues. We also studied the relation between metal content in the soft tissues of the bivalve organisms and in the sediments they inhabit.

2. Material and Methods *Area of study*

The study area constitutes a small part of the low lands lying west of Port Said City known as El-Gamil zone, which extends further west-wards parallel to the Deltaic Coast of the Mediterranean Sea. It is bordered by the Suez Canal to the east and by Lake Manzala to the south. It is situated about 13 kilometers west of Port-Said City and extends between latitude 31°:10' - 31°:20' N and longitude $32^{\circ}:00' - 32^{\circ}:20'$ E with about a 24 km² coverage area. Five sites along El- Gamil beach, including El-Debba (site 5); El-Manasra (site 4); El-Fardous (site 3); El-Gamil inlet (site 2) and the El-Gamil airport (site 1) were chosen for this study (Figure 1). Levels of six heavy metals (Fe, Mn, Cd, Zn, Cu and Pb) were measured in bottom sediment, surface water and the muscle tissues of the bivalve samples collected from each site during October 2006.



Figure1. Satellite image shows the location of the study area and the sampling sites on the Mediterranean Sea.

Analytical procedure Bottom sediment

A total of 30 sediment samples were collected from the five selected sites along the western coast of Port Said (El-Gamil zone) during October 2006. The sediment samples were collected by pushing a plastic core (6 cm in diameter) into the bottom sediment to a depth of approximately 5 cm. The samples were then put in plastic bags and transferred to the laboratory. In the laboratory sediment samples were air-dried (Thomas et al., 1994) and sieved with a 63-µm nylon mesh, and the fraction < 63 µm was chosen for chemical analysis. A pooled sample was prepared for each station by mixing and homogenizing the < 63 µm of the sediment from each station. The samples underwent acid digestion (HNO₃-HCIO₄) in an automatic microwave digestion system and their metal content was analysed by AAS (Usero et al., 2005).

Surface water

In tandem with the collection of the 30 sediment samples a further 30 surface water samples were collected at a depth of 0.5 m from the same five selected sites. Water samples were collected in one liter white polyethylene bottles, and filtered through a 0.45 μ m membrane. The filtered samples were placed in an ice-box, and transferred to the laboratory for storage at 4 °C until analysis. The concentration of the following heavy metals: Fe, Mn, Zn, Cu, Pb and Cd were determined by digesting 100 ml of each water sample at 100 °C in 5 ml analar grade nitric acid (HNO₃) for 5 hours. The digested samples were allowed to stand over–night at room temperature before the residual being analyzed for heavy metals according to Standard Method 3110 (APHA, 1992).

Organisms Spec

Specimens of the most commercial bivalve D. trunculus were collected from the five sampling sites at the same time as the sediment and water samples during October 2006, a month in which the bivalve species has no reproductive activity. The individuals were selected for a standard shell size (25 \pm 5 mm). At each station 30 individuals were collected to prepare a pooled sample to reduce individual variations in heavy metal concentrations (Daskalakis, 1996). After collection, the clams were allowed to flush out undigested matter in filtered seawater from the sampling sites for 24 h (Sokolowski, et al., 2002). The soft tissues of 30 individuals from each location were carefully removed by shelling the bivalves with a plastic knife; they were then freeze-dried (Chiu et al., 2000) and ground to a fine powder in a mortar before analysis (Ruelas-Inzunza and Paez-Osuna, 2000). The resulting powder underwent microwave acid (HNO3 Suprapure) digestion (Camusso et al., 2001). Following acid digestion, all the samples were analyzed for six elements by atomic absorption spectrometry (AAS).

The total concentrations of Fe, Mn, Zn, Cu, Pb and Cd metals in bottom sediment, surface water and the muscle tissue of the bivalve (*D. trunculus*) samples were analyzed using an atomic absorption spectrophotometer (AAS) (Perkin Elmer, Waltham, MA, USA, model 1200 A), at the El-Fostat Center, Cairo, Egypt, according to the Standard Method 3110 (APHA, 1992). The detection limits of the AAS used were $0.002\mu g/l$ for Fe; $0.001 \mu g/l$ for Zn and 0.0005 for Mn, Cu, Pb and Cd, respectively. All analyses were carried out in triplicate. For each run, three "blanks" were analyzed using the same procedure in order to check the purity of reagents and any possible contamination.

Statistical analysis

One-way analysis of variance test (Underwood, 1981) was followed. The significance levels of 5% (Steel and Torrie, 1986) were conducted on each metal to test for significant differences between sites. Sand–metal, silt-metal and interelemental relationships were performed through Pearson's correlation coefficient matrix. All statistical analyses were conducted using the Office Excel 2003 software package.

In addition to determine the level of metals in the edible bivalve *D. trunculus* soft tissues, the concentration factor values (C_f) for the six studied metals were calculated in the soft tissues of the bivalve according to the following formula:

$$\mathbf{C}_f = \mathbf{C}_x / \mathbf{C}_w$$

Where: C_x and C_w are the mean concentrations of metals in the organism, and in the surrounding water, respectively.

To compare the total metal content at the different sampling sites, the metal pollution index (MPI) was used, obtained with the equation (Usero et al., 1997):

 $MPI = (Cf_1 \times Cf_2 \dots Cf_n)^{1/n}$

where $Cf_n = concentration of the metal n in the sample.$

To evaluate the efficiency of metal bioaccumulation in the bivalve species, the biosediment accumulation factor (BSAF), which is defined as the ratio between the metal concentration in the organism and that in the sediment was calculated (Lau et al., 1998; Szefer et al., 1999).

3. Results

The concentration of heavy metals in the sediment samples was recorded in Table 1. In the sediment samples the heavy metals distribution followed the decreasing order of Fe> Mn> Zn> Pb> Cu> Cd. Classification of pollution level in relation to each element was determined according to the rules for sediment pollution adopted by the Ontario Ministry of the Environment (OME) and the United States Environmental Protection Agency (EPA). The classifications -in respect to the pollution levels found in this study are given in Table 2. The concentration of heavy metals in the surface water samples collected during the present study was recorded in Table 3. The

concentration of heavy metals ($\mu g g^{-1} dry wt$) in the soft tissue of *D. trunculus* is shown in Table 5. Moreover, the concentration factor values for the studied metals in *D. trunculus* are delineated in Table 3. The concentration of heavy metals in the bivalve samples showed the decreasing order of Fe> Zn> Pb> Mn> Cu> Cd. Cd and Zn are the metals with the highest mean of biosediment accumulation factor (BSAF) in the soft tissues of *D. trunculus*; followed by Cu (Table 5). Various degrees of correlations were found between the elements, sand, and silt (Table 5).

Table 1. Metal concentrations ($\mu g/g$ dry mass) in sediments

Site	Element					
	Fe	Mn	Cd	Zn	Cu	Pb
(1)	1830	197.2	1.9	30.4	6.6	20.6
(2)	1837.6	202.9	1.4	32.4	5.7	18.8
(3)	1748.2	191.4	1.8	28.0	6.6	20.4
(4)	1888.6	217.8	2.0	36.6	9.4	24.4
(5)	1918.7	208.3	1.9	32.5	7.0	2.1

Table 2. Classification of pollution level (total means :TOM) in μ g/g of each element according to the American rules for sediment, in conformity to the lowest effect level (LEL), heavily polluted category (HPC) and severe effect level (SEL), (modified from Freret-Meurer et al., 2010).

Metals	TOM	LEL	HPC	SEL
Fe	1895.15	20000	25000	40000
Mn	211.98	460	500	1100
Cd	1.94	0.6	6	10
Zn	34.06	-	-	-
Cu	6.83	16	50	110
Pb	21.52	31	60	250

Table 3. Metal concentrations ($\mu g/g$ dry mass) in water, mean values, and concentration factor values (C_f) for the six studied metals.

Site	Element							
	Fe	Mn	Cd	Zn	Cu	Pb		
(1)	872.6	198	2.6	300	13.3	42.6		
(2)	832	190.6	0.8	271.3	12.6	40.0		
(3)	822.6	180	2.7	283.3	14	41.3		
(4)	846.6	169.3	2.6	294.6	19.3	50.6		
(5)	896	197.3	2.4	282.6	18.6	56.0		
Mean	853.96	187.04	2.22	286.36	15.56	46.1		
C_f	71.55	36.58	1209.82	119.03	278.61	198.6		

4. Discussions

The concentration of heavy metals in the sediment samples was recorded in (Table 1). Even though there was variability among sites, the overall concentration range for a particular metal was relatively narrow, with no values that appeared to be unusual. Generally, in the sediment samples the heavy metals distribution followed the decreasing order of Fe> Mn> Zn> Pb> Cu> Cd. Site (4) demonstrated the highest level of heavy metal contamination recorded during the present study. This can be explained by the increasing industrial activities at this site, with natural gas companies, pipeline industries and an electric power generating station all operating in this area.

Classification of pollution level in relation to each element was determined according to the rules for sediment pollution adopted by the Ontario Ministry of the Environment (OME) and the United States Environmental Protection Agency (EPA). On this scale, pollution is categorized as being, respectively, at the lowest effect level (LEL), the heavily polluted category (HPC) and the severe effect level (SEL). The classifications -in respect to the pollution levels found in this study are given in Table 2. Judging by the present results, El-Gamil beach at the western coast of Port Said on the Mediterranean Sea can be considered a non-metal-polluted area, according to the OME and EPA classification criteria. These observations agree with those found by El-Sikaily et al. (2004) at other Egyptian coastal areas on the Mediterranean and Red Sea.

The concentration of heavy metals in the surface water samples collected during the present study was recorded in (Table 3). As with the sediment samples, even though there was variability among sites, the overall concentration range for a particular metal was relatively narrow, with no values that appeared to be unusual. Not surprisingly, Fe concentrations were the highest, ranging from 822.6 \pm 14.7 µg g⁻¹ at El Fardous (site 3), to 896 \pm 15 µg g⁻¹ at El-Debba (site 5). Cd concentrations were the lowest and ranged from 0.8 \pm 1.3 µg g⁻¹ at El-Gamil inlet (site 2) to 2.7 \pm 0.11 µg g⁻¹ at El-Fardous (site 3). Similar values were recorded by Kaiser et al. (2010).

The concentration of heavy metals ($\mu g g^{-1} dry$ wt) in the soft tissue of D. trunculus is shown in Table 4. The concentration of heavy metals in the bivalve samples showed the decreasing order of Fe> Zn> Pb> Mn> Cu> Cd. Similar findings were given by Hornung and Oren (1981) and Abdallah and Abdallah (2008). Based on the data given in Table 5, it seems that the observed variation in metal levels in D. trunculus at different sites can be attributed to two mechanisms. The first is the availability of the metals in different sites, which in its turn depends on the pollution sources that of course vary across the sites. The second is that the animal utilizes different uptake and retention mechanisms which may also vary with physiological and environmental factors (Bryan, 1973) or even with the sexual state of the animal (Alexander and Young, 1976). Generally, however, the values of heavy metal concentration varied within an insignificant range in summer season and across

the sampling sites due to similar conditions affecting these sites. The highest concentration level of Zn $(36.4 \pm 3.8 \ \mu g \ g^{-1})$ and Cu $(4.4 \pm 0.3 \ \mu g \ g^{-1})$ metals in the analyzed species are less than the maximum permissible levels (MPLs) of 100 μ g g⁻¹ and 10 μ g g⁻¹ for Zn and Cu, respectively. The (MPLs) of 2 $\mu g g^{-1}$, and 5 μ g g⁻¹ for Cd and Pb (declared by WHO 1982; FAO 1983; FAO/WHO 1987; WHO 2006), as well as maximum levels of Cd and Pb of 1.0 and 1.5 µg g⁻¹, respectively declared for bivalve mollusks by Commission Regulation (EC) (EU 2006) are, however, much lower than those detected in the soft tissues of D. trunculus with maximum values of 2.4 \pm 0.3 μ g g⁻¹ for Cd and 9.2 \pm 0.2 μ g g⁻¹ for Pb. D. trunculus inhabiting El-Gamil beach along the western coast of Port Said on the Mediterranean Sea, therefore, is, in this respect, likely to be toxic for public health. Judging by the results of the present study the examined bivalve species (D. trunculus), was associated with enhanced metal content in its soft tissues and was unsafe within the limits for human consumption.

Table 4. Metal concentrations (μ g/g dry mass), mean values, metal pollution index (MPI), and mean biosediment accumulation factor values (BSAF) in *D. trunculus*.

Site	Element								
	Fe	Mn	Cd	Zn	Cu	Pb	MPI		
(1)	66.4	7.6	2.0	36.4	4.4	9.2	10.7		
(2)	61.8	6.8	2.2	34.8	4.2	9.0	10.3		
(3)	57.2	6.0	2.4	32.8	4	8.8	9.9		
(4)	60.7	7.1	2.1	33.4	4.3	9.0	10.2		
(5)	59.3	6.3	2.1	32.9	4.1	8.9	9.9		
Mean	61.08	6.76	2.16	34.06	4.2	8.98			
BSAF	0.033	0.032	1.22	1.07	0.60	0.42			

The concentration factor of metals is also considered to be an indicator of heavy metal accumulation in the tissues of aquatic organisms when expressed in relation to their concentration in the ambient water (Sultana and Rao, 1998). The concentration factor values for the studied metals in *D. trunculus* are delineated in Table 3. For *D*. trunculus, Cd gave the highest accumulation rate in the animal tissue with C_f values of 1209.82. The order of C_f in the soft tissues of *D. trunculus* was Cd> Cu> Pb>Zn>Fe>Mn, respectively. Thus, these patterns of magnitude were changed when the order carried out according to the level of heavy metals concentration in the soft tissues of the bivalve species, which were Fe> Zn> Pb> Mn> Cu> Cd. Thus, Cd and Cu moved from the end of the concentration pattern and occupied the top of the accumulation pattern (C_{f}). This phenomenon indicates that the studied species D. trunculus has a bioavailability to accumulate Cd and Cu from the surrounding medium greater than other metals and can suggest using of D. trunculus as a good indicator for the presence of highly toxic metals such as Cd and as a bio-indicator for essential metals such as Cu. Increased Cd levels in the bivalve are worrying, especially considering the fact that it could be one of the most toxic heavy metals, even at relatively low concentrations (Fianko et al., 2007).

Cd and Zn are the metals with the highest mean of biosediment accumulation factor (BSAF) in the soft tissues of *D. trunculus*; followed by Cu (Table 4). Similar findings were recorded by Usero et al., (2005) for *D. trunculus* from the Atlantic coast of southern Spain. Generally *D. trunculus* has a great capacity for metal bioaccumulation with particular reference to highly toxic metal such as Cd. The concentration of most of the metals in the bivalve *D. trunculus* varied notably depending on the location of the sampling sites. Table 5 shows the metal pollution index (MPI) for this species increased at sites 1, 2 and 4 which are characterized by different anthropogenic sources of heavy metals contamination.

Statistically, significance analysis (p values from the ANOVA-test) was performed between sites 1, 2, 3, 4, and 5. Although, there were differences in heavy metals concentrations between the different five sites, these differences in heavy metal concentration were not statistically significant (p > 0.05). Thus there is no heavy metal adding source in any of the five sites chosen for the present study.

	Fe	Mn	Cd	Zn	Cu	Pb	Sand %	Silt %
Fe	1							
Mn	0.85	1						
Cd	0.29	0.31	1					
Zn	0.78	0.98	0.23	1				
Cu	0.43	0.75	0.74	0.72	1			
Pb	0.43	0.71	0.79	0.68	0.9	1		
Sand %	0.24	0.16	0.96	0.02	0.56	0.6	1	
Silt %	-0.3	-0.2	-0.9	-0.04	-0.5	-0.6	-0.9	1

Table 5. Correlation coefficient matrix between heavy metals, silt and sand grains in sediments.

Various degrees of correlations were found between the elements, sand, and silt (Table 5). The correlation coefficient matrix between heavy metal concentrations and the physico-chemical characteristics of the sediment samples of El-Gamil beach showed some significant correlations, both positive and negative. There was positive relationships (p < 0.05) between the six elements; sand and Fe; sand and Mn; sand and Cd; sand and Cu; sand and Pb. Moreover, an inverse significant correlations were noticed between silt and Fe, silt and Mn; silt and Cd; silt and Cu; silt and Pb; silt and sand, and finally no significant correlations were found (p > p)0.05) between sand and Zn. Judging by the results, the six elements measured in the present study clearly have similar anthropogenic sources; and having similar sources of course can be related to the specific geographical structure of this area.

In conclusion, this study was carried out to obtain quantitative information on the concentration of heavy metals in the edible bivalve D. trunculus from the Egyptian coasts of Port Said on the Mediterranean. Natural gas companies, pipeline industries and an electric power generating station are the main anthropogenic sources of heavy metals contamination in this region. Some results were above the limits for mollusks proposed by FAO/WHO, 1987 and EU, 2006. The present study has revealed that the concentrations of Cd and Pb in the soft tissues of the edible bivalve D. trunculus were above the maximum acceptable concentrations for human consumption. According to our results, the examined bivalve species D. trunculus was associated with enhanced metal content in its soft tissues and was unsafe within the limits for human consumption. Although, levels of heavy metals in water and sediment are not high, a potential danger may emerge in the future depending on the domestic waste waters and agricultural activities in this region. We conclude that, the area of the present study is in general not considered a metal polluted area according to the OME and EPA classification criteria. However, concentrations of Cd and especially, Pb did give some cause for concern, and warrants a continued monitoring programme for inorganic and chemical organic compounds in sediments, water, and biota along the Egyptian Mediterranean coasts. The bivalve D. trunculus populations clearly have significant potential as useful bioindicators in the assessment of heavy metal pollution along a coastal area of Port Said on the Mediterranean Sea.

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